Human pluripotent stem cells: the development of high-content screening strategies.

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Authors: Sean P Sherman, Jackelyn A Alva, Kaushali Thakore-Shah, April D Pyle

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Public Summary:
High-content screening (HCS) permits simultaneous observation and analysis of multiple cellular variables including cell morphology, survival, and differentiation in live cells at the single-cell level, at the level of the culture well, and across the entire culture. By combining high-throughput technologies such as robotics, chemical libraries, and automated high-resolution microscopy, scientists are able to evaluate a much broader array of experimental conditions than can be studied using conventional cell biological techniques that study fewer parameters at any one time. Thus, HCS assays provide a means to vastly improve our basic understanding of stem cell biology. We have developed a HCS assay that allows the study of the effects of hundreds of small molecules in parallel. The protocol described in this chapter was developed to assess the effects of small molecules on the survival, proliferation, and expression of pluripotent markers following single-cell dissociation of human embryonic stem cells, but can be applied to the study of other types of stem cells including induced pluripotent stem cells. A detailed protocol for the setup of HCS assays and the parameters used to identify chemical modifiers of survival in human pluripotent stem cells, as well as secondary assays used to validate the small-molecule "hits" obtained during the high-content screen, are described.

Scientific Abstract:
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